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EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to soy isoflavones and protection of DNA, proteins and lipids from oxidative damage (ID 1286, 4245), maintenance of normal blood LDL-cholesterol concentrations (ID 1135, 1704a, 3093a), reduction of vasomotor symptoms associated with menopause (ID 1654, 1704b, 2140, 3093b, 3154, 3590), maintenance of normal skin tonicity (ID 1704a), contribution to normal hair growth (ID 1704a, 4254), "cardiovascular health" (ID 3587), treatment of prostate cancer (ID 3588), and "upper respiratory tract" (ID 3589) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to soy isoflavones and protection of DNA, proteins and lipids from oxidative damage (ID 1286, 4245), maintenance of normal blood LDL-cholesterol concentrations (ID 1135, 1704a, 3093a), reduction of vasomotor symptoms associated with menopause (ID 1654, 1704b, 2140, 3093b, 3154, 3590), maintenance of normal skin tonicity (ID 1704a), contribution to normal hair growth (ID 1704a, 4254), "cardiovascular health" (ID 3587), treatment of prostate cancer (ID 3588) and "upper respiratory tract" (ID 3589) pursuant to Article 13(1) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to soy isoflavones and protection of DNA, proteins and lipids from oxidative damage, maintenance of normal blood LDL-cholesterol concentrations, reduction of vasomotor symptoms associated with menopause, maintenance of normal skin tonicity, contribution to normal hair growth, "cardiovascular health", treatment of prostate cancer, and "upper respiratory tract". The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food constituent that is the subject of the health claims is soy isoflavones. The Panel considers that soy isoflavones are sufficiently characterised.

Protection of DNA, proteins and lipids from oxidative damage

The claimed effects are "vascular effects including protection from oxidative damage" and "antioxidant status". The target population is assumed to be the general population. In the context of the proposed wordings, the Panel assumes that the claimed effects refer to the protection of body cells and molecules from oxidative damage. The Panel considers that protection of DNA, proteins and lipids from oxidative damage may be a beneficial physiological effect.

No human studies from which conclusions could be drawn for the scientific substantiation of the claim were provided.

The Panel concludes that a cause and effect relationship has not been established between the consumption of soy isoflavones and protection of DNA, proteins and lipids from oxidative damage.

Maintenance of normal blood LDL-cholesterol concentrations

The claimed effects are "cholesterol management / heart health", "menopause/skin and hair health during menopause/cholesterol management" and "act as phyto-estrogens". The target population is assumed to be the general population. In the context of the proposed wordings and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the maintenance of normal blood LDL-cholesterol concentrations. The Panel considers that maintenance of normal blood LDL-cholesterol concentrations is a beneficial physiological effect.

The Panel notes that one meta-analysis including nine RCTs, and three additional RCTs, did not show an effect of extracted soy isoflavones on blood LDL-cholesterol concentrations. The Panel also notes that out of the 23 human intervention studies in which soy isoflavones were consumed in soy protein from which conclusions could be drawn for the scientific substantiation of the claim, 14 studies (n=1,286 subjects, 12-93 subjects per group/period) with durations between one and 12 months using isoflavone doses of around 60-330 mg per day did not show an effect of soy isoflavones on blood cholesterol concentrations in subjects with slightly elevated or high blood LDL-cholesterol concentrations, whereas eight studies (n=712 subjects, 15-42 subjects per group/period) with durations between six weeks and six months using isoflavone doses of around 30-185 mg per day showed a statistically significant effect in subjects with normal to high blood LDL-cholesterol concentrations, and one study (n=94 subjects, around 30 per group) led to inconsistent results with respect to the effect of soy isoflavones on blood LDL-cholesterol concentrations. The Panel also notes that most of the studies had some methodological limitations, and that the inconsistent results reported appear unrelated to the dose of isoflavones used, the study duration, the sample size or the baseline characteristics of subjects with respect to blood cholesterol concentrations.

In weighing the evidence, the Panel took into account that one meta-analysis of nine randomised controlled trials, and three additional randomised controlled trials, did not show an effect of extracted soy isoflavones on blood cholesterol concentrations, and that the evidence provided by 23 human randomised controlled trials in which soy isoflavones were consumed in soy protein is inconsistent.

The Panel concludes that a cause and effect relationship has not been established between the consumption of soy isoflavones and maintenance of normal blood LDL-cholesterol concentrations.

Reduction of vasomotor symptoms associated with menopause

The claimed effects are "menopause", "menopause/skin and hair health during menopause/cholesterol management", "soy contains the phytoestrogens isoflavones that can function as either an estrogen agonist or antagonist", "act as phytoestrogens", "helps to keep healthy thermoregulation during

climacterium", and "helps to alleviate the symptoms of menopause". The target population is assumed to be post-menopausal women. In the context of the proposed wordings and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the reduction of vasomotor symptoms associated with menopause. The Panel considers that reduction of vasomotor symptoms associated with menopause is a beneficial physiological effect.

The Panel notes that from the 12 human intervention studies provided from which conclusions can be drawn for the scientific substantiation of the claim, most of which have some methodological limitations and/or inadequate reporting, five studies (n=498 subjects analysed; range: 25-115 per group) with a duration of 12 weeks to 12 months using doses from 27 mg genistein to 100 mg of total isoflavones showed a statistically significant effect of soy isoflavones on vasomotor symptoms after one month (one study), three months (two studies), four months (one study) and 10 months (one study), whereas six studies (n=463 subjects analysed, range: 12-99 per group/period) with a duration of six weeks to six months using doses from 40 mg to 118 mg of total isoflavones did not show a statistically significant effect of soy isoflavones on vasomotor symptoms, and that one study in 122 analysed subjects using a dose of 50 mg isoflavones for four months led to inconsistent results with regard to the effect soy isoflavones on vasomotor symptoms.

In weighing the evidence, the Panel took into account that the evidence provided by 12 human intervention studies is inconsistent with respect to the reduction of vasomotor symptoms, and that the inconsistent results cannot be explained by dose, sample size, study duration, or baseline frequency or severity of vasomotor symptoms.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of soy isoflavones and reduction of vasomotor symptoms associated with menopause.

Maintenance of normal skin tonicity

The claimed effect is "menopause/skin and hair health during menopause/cholesterol management". The target population is assumed to be post-menopausal women. In the context of the proposed wordings and the clarifications provided by Member States, the Panel assumes that the claimed effect refers to the maintenance of normal skin tonicity. No evidence has been provided on how skin tonicity could be related to skin function.

The Panel considers that the claim does not refer to a function of the body as required by Regulation (EC) No 1924/2006.

Contribution to normal hair growth

The claimed effect is "menopause/skin and hair health during menopause/cholesterol management" and "hair growth and loss". The target population is assumed to be the general population. In the context of the proposed wordings, the Panel assumes that the claimed effects refer to normal hair growth. The Panel considers that contribution to normal hair growth is a beneficial physiological effect.

No references from which conclusions could be drawn for the scientific substantiation of the claim were provided.

The Panel concludes that a cause and effect relationship has not been established between the consumption of soy isoflavones and contribution to normal hair growth.

"Cardiovascular health"

The claimed effect is "contributes to cardiovascular health". The target population is assumed to be the general population. The claimed effect is not sufficiently defined and no further details were provided in the proposed wordings. No clarifications were provided by Member States.

The Panel considers that the claimed effect is general and non-specific, and does not refer to any specific health claim as required by Regulation (EC) No 1924/2006.

Treatment of prostate cancer

The claimed effect is "contributes to maintain a healthy prostate and breast". The target population is assumed to be the general population. From the references provided, it is assumed that the claimed effect is related to the treatment of prostate cancer.

The Panel considers that the claim is related to the treatment of disease and does not comply with the criteria laid down in Regulation (EC) No 1924/2006.

"Upper respiratory tract"

The claimed effect is "contributes to the upper respiratory tract health". The target population is assumed to be the general population. The claimed effect is not sufficiently defined and no further details were provided in the proposed wordings. No clarifications were provided by Member States.

The Panel considers that the claimed effect is general and non-specific, and does not refer to any specific health claim as required by Regulation (EC) No 1924/2006.

KEY WORDS

Soy isoflavones, oxidative damage, cholesterol, skin tonicity, hair growth, vasomotor symptoms, menopause, prostate cancer, cardiovascular health, upper respiratory tract, health claims.

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INFORMATION AS PROVIDED IN THE CONSOLIDATED LIST

The consolidated list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006[4] submitted by Member States contains main entry claims with corresponding conditions of use and literature for similar health claims. EFSA has screened all health claims contained in the original consolidated list of Article 13 health claims which was received by EFSA in 2008 using six criteria established by the NDA Panel to identify claims for which EFSA considered sufficient information had been provided for evaluation and those for which more information or clarification was needed before evaluation could be carried out[5]. The clarifications which were received by EFSA through the screening process have been included in the consolidated list. This additional information will serve as clarification to the originally provided information. The information provided in the consolidated list for the health claims which are the subject of this opinion is tabulated in Appendix C.

ASSESSMENT

1.   Characterisation of the food/constituent

The food constituent that is the subject of the health claims is soy isoflavones.

Soy isoflavones constitute a wide range of compounds of plant origin, which mainly comprise genistein, daidzein and glycitein, among others (Ma et al., 2008a, 2008b). Soy isoflavones can be consumed as isolated soybean protein (ISP), as whole-soybean foods or extracts, as supplements or as pure compounds (Cassidy et al., 2006).

The Panel considers that the food constituent, soy isoflavones, which is the subject of the health claims, is sufficiently characterised.

2.   Relevance of the claimed effect to human health

2.1.   Protection of DNA, proteins and lipids from oxidative damage (ID 1286, 4245)

The claimed effects are "vascular effects including protection from oxidative damage" and "antioxidant status". The Panel assumes that the target population is the general population.

The Panel considers that claims made on the antioxidant capacity/content or properties of foods/food constituents based on their capability to scavenge free radicals *in vitro* refer to a property of the foods/food constituents measured in model systems, and that the information provided does not establish that this capability exerts a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.

In the context of the proposed wordings, the Panel assumes that the claimed effects refer to the protection of body cells and molecules from oxidative damage caused by free radicals.

Reactive oxygen species (ROS) including several kinds of radicals are generated in biochemical processes (e.g. respiratory chain) and as a consequence of exposure to exogenous factors (e.g. radiation and pollutants). These reactive intermediates can damage molecules such as DNA,

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[4] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

[5] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011. General guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims. EFSA Journal, 9(4):2135, 24 pp.

proteins and lipids if they are not intercepted by the antioxidant network which includes free radical scavengers such as antioxidant nutrients.

The Panel considers that protection of DNA, proteins and lipids from oxidative damage may be a beneficial physiological effect.

2.2. Maintenance of normal blood LDL-cholesterol concentrations (ID 1135, 1704a, 3093a)

The claimed effects are "cholesterol management / heart health", "menopause/skin and hair health during menopause/cholesterol management" and "act as phyto-estrogens". The Panel assumes that the target population is the general population.

In the context of the proposed wordings and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the maintenance of normal blood LDL-cholesterol concentrations.

Low-density lipoproteins (LDL) carry cholesterol from the liver to peripheral tissues, including the arteries. Elevated LDL-cholesterol, by convention >160 mg/dL (>4.1 mmol/L), may compromise the normal structure and function of the arteries.

The Panel considers that maintenance of normal blood LDL-cholesterol concentrations is a beneficial physiological effect.

2.3. Reduction of vasomotor symptoms associated with menopause (ID 1654, 1704b, 2140, 3093b, 3154, 3590)

The claimed effects are "menopause", "menopause/skin and hair health during menopause/cholesterol management", "soy contains the phytoestrogens isoflavones that can function as either an estrogen agonist or antagonist", "act as phytoestrogens", "helps to keep healthy thermoregulation during climacterium", and "helps to alleviate the symptoms of menopause". The Panel assumes that the target population is post-menopausal women.

In the context of the proposed wordings and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the reduction of vasomotor symptoms associated with menopause. Changes in vasomotor symptoms associated with menopause such as frequency and severity of hot flushes and night sweats can be assessed using questionnaires.

The Panel considers that reduction of vasomotor symptoms associated with menopause is a beneficial physiological effect.

2.4. Maintenance of normal skin tonicity (ID 1704a)

The claimed effect is "menopause/skin and hair health during menopause/cholesterol management". The Panel assumes that the target population is post-menopausal women.

In the context of the proposed wordings and the clarifications provided by Member States, the Panel assumes that the claimed effect refers to the maintenance of normal skin tonicity. No evidence has been provided on how skin tonicity could be related to skin function.

The Panel considers that the claim does not refer to a function of the body as required by Regulation (EC) No 1924/2006.

2.5.   Contribution to normal hair growth (ID 1704a, 4254)

The claimed effect is "menopause/skin and hair health during menopause/cholesterol management" and "hair growth and loss". The Panel assumes that the target population is the general population.

In the context of the proposed wordings, the Panel assumes that the claimed effects refer to normal hair growth.

The Panel considers that contribution to normal hair growth is a beneficial physiological effect.

2.6.   "Cardiovascular health" (ID 3587)

The claimed effect is "contributes to cardiovascular health". The Panel assumes that the target population is the general population.

The claimed effect is not sufficiently defined and no further details were provided in the proposed wordings. No clarifications were provided by Member States.

The Panel considers that the claimed effect is general and non-specific, and does not refer to any specific health claim as required by Regulation (EC) No 1924/2006.

2.7.   Treatment of prostate cancer (ID 3588)

The claimed effect is "contributes to maintain a healthy prostate and breast". The Panel assumes that the target population is the general population.

In the context of the proposed wordings, the Panel assumes that the claimed effect refers to prostate function. Prostate function is not sufficiently defined. From the references provided, the Panel assumes that the claimed effect is related to the treatment of prostate cancer.

The Panel considers that the claim is related to the treatment of disease and does not comply with the criteria laid down in Regulation (EC) No 1924/2006.

2.8.   "Upper respiratory tract" (ID 3589)

The claimed effect is "contributes to the upper respiratory tract health". The Panel assumes that the target population is the general population.

The claimed effect is not sufficiently defined and no further details were provided in the proposed wordings. No clarifications were provided by Member States.

The Panel considers that the claimed effect is general and non-specific, and does not refer to any specific health claim as required by Regulation (EC) No 1924/2006.

3.   Scientific substantiation of the claimed effect

3.1.   Protection of DNA, proteins and lipids from oxidative damage (ID 1286, 4245)

Among the references provided were a number of narrative reviews and textbooks which were either not related to the claimed effect or did not provide any original data which could be used for the scientific substantiation of the claim. A number of human, animal and *in vitro* studies were not related to the claimed effect; these included references on endothelial function, antioxidant gene expression, metabolic syndrome, blood pressure, cholesterol concentrations, subarachnoid haemorrhage,

cardiovascular disease, atherosclerosis and symptoms associated with menopause. Some of the references provided did not address the food constituent which is the subject of the claim, but rather soy foods in general, or soy foods in combination with other foods. These studies did not control for other components besides isoflavones in these soy foods, which could have an effect on the protection of DNA, proteins and lipids from oxidative damage. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

Human intervention studies which assessed the effects of soy isoflavones on plasma total antioxidant status (DiSilvestro et al., 2006) and on *ex vivo* resistance of LDL to peroxidation (Jenkins et al., 2002; Nestel et al., 1997; Steinberg et al., 2003), and *in vitro* studies which assessed the effect of soy isoflavones on the oxidation lag time of LDL particles (Hwang et al., 2000) and on the resistance of HDL to copper-ion-induced peroxidation by measuring conjugated dienes (Ferretti et al., 2004) were provided. The Panel notes that the evidence provided does not establish that these markers predict peroxidation of LDL or HDL particles *in vivo* (Griffiths et al., 2002; Lapointe et al., 2006; Verhoye and Langlois, 2009). The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

A randomised, double-blind, cross-over study by Wiseman et al. (2000) investigated the effects of diets low or high in soy isoflavones on lipid peroxidation in 24 healthy subjects aged 19-40 years. The subjects consumed textured soy protein as vegetarian burgers high (21.2 mg daidzein and 34.8 mg genistein) or low (0.9 mg daidzein and 1.0 mg genistein) in isoflavones for 17 consecutive days each, separated by a 25-day wash-out period. Two subjects did not complete the low-isoflavone intervention. Blood plasma concentrations of 8-epiprostaglandin $F_{2\alpha}$ (8-epi-PGF$_{2\alpha}$), and malondialdehyde (MDA) measured by the HPLC-based thiobarbituric acid test, and resistance of LDL to copper-ion-induced peroxidation were assessed in 18 samples for each intervention period. The statistical analysis, which was corrected for multiple comparisons, was based on these samples only. The Panel notes the use of a valid biomarker to assess lipid peroxidation (8-epi-PGF$_{2\alpha}$ determined by gas chromatography–mass spectrometry) and the inclusion of a marker which could be used as supportive (MDA measured by HPLC). The Panel notes that resistance of LDL to copper-ion-induced peroxidation is not a reliable marker to assess lipid peroxidation *in vivo*. The Panel also notes that 25 % of the samples were not available for analysis, and that missing data were not taken into account in data analysis, and considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

The Panel notes that no human studies from which conclusions could be drawn for the scientific substantiation of the claim were provided.

The Panel concludes that a cause and effect relationship has not been established between the consumption of soy isoflavones and protection of DNA, proteins and lipids from oxidative damage.

3.2. Maintenance of normal blood LDL-cholesterol concentrations (ID 1135, 1704a, 3093a)

Among the references provided were a number of narrative reviews and textbooks which were either not related to the claimed effect or did not provide any original data which could be used for the scientific substantiation of the claim. A number of human, animal and *in vitro* studies did not address changes in LDL-cholesterol concentrations; these included references on the effect of soy isoflavones on symptoms associated with menopause, severity of asthma, DNA damage, mood, cognitive function, bone loss, bone fractures, cancer, LDL peroxidation, weight loss, inflammation, cardiovascular disease risk, deposition of cholesterol in the aorta and endothelial function. Some of the references provided did not address the food constituent, which is the subject of the claim, but addressed soy isoflavones in combination with other substances, or red clover isoflavones.

Fifty-five human intervention studies were provided either separately in the consolidated list or in the four meta-analyses which assessed the effect of soy isoflavones as extracts, pure compounds or in soy protein on blood cholesterol concentrations.

Some human intervention studies used soy nuts (Welty et al., 2007b), soy foods in general (Chiechi et al., 2002; Scheiber et al., 2001), soy meal replacement formula (Allison et al., 2003), soy protein containing isoflavones (Maesta et al., 2007; Sagara et al., 2004; Washburn et al., 1999) or a soy extract, fibre and lecithin supplement (Puska et al., 2002), without controlling either for the protein component, the amount of lecithin contained or the macronutrient composition, which could have an effect on blood cholesterol concentrations. In one study (Takatsuka et al., 2000), the nature of the control diet was not specified. Two studies, one of which was uncontrolled, did not report the amount of isoflavones administered (Wong et al., 1998; Yildirir et al., 2001). One study examined the effect of soy isoflavones on post-prandial cholesterol concentrations only (Campbell et al., 2006), one study assessed total cholesterol concentrations without reporting on LDL-cholesterol or other cholesterol fractions (Urban et al., 2001), and one study purported to have evaluated blood cholesterol concentrations but did not report any results (Scambia et al., 2000). In four studies (Basaria et al., 2009; Jenkins et al., 2000; Jenkins et al., 2002; West et al., 2005) some subjects took medication such as hormone replacement therapy (HRT), levothyroxine or cholesterol lowering medication which could have an impact on the claimed effect, and it was unclear whether the sub-group analysis performed in subjects not taking medication in one of these studies (West et al., 2005) was pre-planned or not. In one study, subjects who dropped out were replaced after randomisation (Lichtenstein et al., 2002), introducing possible selection bias. In a further study, subjects in the intervention and placebo groups were significantly different with respect to their total and LDL-cholesterol concentrations at baseline, and it was unclear how this difference was taken into account in the analysis (Petri Nahas et al., 2004). Three cross-sectional studies (de Kleijn et al., 2002; Nagata et al., 1998; Somekawa et al., 2001) were provided which investigated the association between soy consumption and changes in blood LDL-cholesterol concentrations. The Panel notes that the observational study design does not allow controlling with sufficient precision for the macronutrient composition of the diets and medication use, both of which could have an effect on blood cholesterol concentrations, besides isoflavones. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

A meta-analysis of randomised controlled trials (RCTs) (Taku et al., 2008), which assessed the effects of consumption of extracted soy isoflavones *vs.* placebo on blood LDL-cholesterol concentrations, included the individual studies of this type which were provided in the consolidated list (Cheng et al., 2007; Gonzalez et al., 2007) except three (Atteritano et al., 2007; Lissin et al., 2004; Nahas et al., 2007).

In the meta-analysis by Taku et al. (2008), several databases were searched for RCTs published between 1966 and 2007. Reference lists of relevant systematic reviews and meta-analyses were hand searched. Twelve studies met the inclusion criteria of RCTs conducted in adults and published in English, Japanese or Chinese, with duration of 1-3 months. Six trials used a parallel design and the remaining trials used a cross-over design. The study population was post-menopausal women (n=565) in all studies, and doses of total isoflavones were 27-132 mg/day expressed as aglycone equivalents. In most of the studies included, women consumed similar diets with similar amounts of fat, cholesterol and fibre, and no significant body weight changes were reported during the study period. The funnel plots did not indicate publication bias. Using either a random or a fixed (i.e. when studies responsible for significant heterogeneity were removed from analysis) effects model, no effect of isolated soy isoflavone consumption on total (ten studies) or LDL-cholesterol (nine studies) concentrations was observed. The Panel notes that no justification has been provided for excluding studies with a duration longer than three months, and that this meta-analysis does not show an effect of extracted soy isoflavones on blood cholesterol concentrations when consumed for 1-3 months at doses of 27-132 mg/day.

In a randomised double-blind, placebo-controlled parallel study conducted in 389 post-menopausal women (49-67 years), the effect of genistein administration (54 mg/day) for 24 months on total and LDL-cholesterol concentrations was investigated (Atteritano et al., 2007). After a run-in phase of four weeks in which all women followed a fat-restricted diet, women were assigned to consume either genistein (n=198) or placebo (n=191) in the form of capsules. All capsules also contained 500 mg calcium and 400 IU vitamin D. Eighty-five subjects withdrew before completion of the study (48 in the genistein group and 37 in the placebo group). Analyses were performed in the intention-to-treat population. No significant changes in total or LDL-cholesterol concentrations were observed between groups at either 12 or 24 months. The Panel notes that this study does not show an effect of genistein consumption on blood cholesterol concentrations.

In a randomised, double-blind, placebo-controlled parallel study 80 post-menopausal women (mean age approx. 55 years) were allocated to consume daily either 100 mg of isoflavones in 250 mg standardised soy extract (n=40) or placebo (lactose; n=40) for ten months (Nahas et al., 2007). Four subjects dropped out during the study (two in the intervention group (n=38 analysed) and two in the placebo group (n=38 analysed)) and were not taken into account in the analysis. No statistically significant differences were observed between the isoflavone and the placebo group with respect to total or LDL-cholesterol concentrations. The Panel notes that missing data have not been taken into account in the analysis, and that this study does not show an effect of extracted soy isoflavones on blood cholesterol concentrations.

In another randomised, double-blind, placebo-controlled parallel trial, 40 post-menopausal women (mean age 61.6±8.4 years) with moderate hypercholesterolaemia were assigned to consume for six weeks either 2x45 mg/day soy isoflavones in tablet form (n=20) or placebo (n=20), which was identical in composition but isoflavone-free (Lissin et al., 2004). All women completed the study. No significant changes in total or LDL-cholesterol concentrations were observed between groups. The Panel notes that this study does not show an effect of extracted soy isoflavones on blood cholesterol concentrations.

The Panel notes that one meta-analysis including nine RCTs, and three additional RCTs, did not show an effect of extracted soy isoflavones on blood LDL-cholesterol concentrations.

Three meta-analyses and 23 human intervention studies were provided which assessed the effect of soy isoflavones in soy protein against isoflavone-depleted soy protein or animal protein.

The meta-analyses by Zhuo et al. (2004) and Taku et al. (2007) investigated the effects of consumption of soy isoflavones in isolated soy protein (ISP[+]) compared to isoflavone-depleted ISP (ISP[-]) on blood cholesterol concentrations in meta-analyses of RCTs published between 1966 and 2003, and between 1990 and 2006, respectively, with duration of 1-3 months, using either a parallel or cross-over design and which provided baseline and endpoint blood cholesterol concentrations. Eight (n=471 subjects) and eleven (n=441 subjects) studies, respectively, met the inclusion criteria. The Panel notes that with the exclusion of studies comparing ISP[+] to animal protein these meta-analyses did not include all the studies pertinent to the claim, and considers that no conclusions can be drawn from these meta-analyses for the scientific substantiation of the claim.

Zhan and Ho (2005) conducted a meta-analysis of RCTs published in English between 1995 and June 2002 on the effect of ISP[+] or isoflavone extracts on blood lipid concentrations. Twenty-three studies (n=1,381 subjects) met the inclusion criteria (RCTs providing the amount of soy isoflavones and blood lipid concentrations at baseline). The study duration ranged from 3 to 26 weeks. Seventeen studies used ISP[+], three used tablets containing extracted isoflavones, and three used textured soy foods. In sub-group analyses, eight comparisons (n=440) were made for ISP[+] vs. ISP[-], 27 comparisons (n=1,501) for ISP[+] vs. casein, and three comparisons (n=135) for extracted soy isoflavones vs. placebo. The Panel notes that in two studies (Puska et al., 2002; Washburn et al., 1999) the control was not appropriate to assess the effects of soy isoflavones in soy protein (i.e. carbohydrates and no

isoflavone-free control, and the intervention and control not matched with respect to the lecithin content). One study included subjects on HRT, levothyroxin and lovastatin (Jenkins et al., 2000), and one study assessed the effects of red clover isoflavones rather than soy isoflavones (Hodgson et al., 1998). It is also unclear which studies were included in the sub-group analyses with respect to ISP$^+$ *vs.* ISP$^-$, and with respect to ISP$^+$ *vs.* casein, or the reasons for excluding from the sub-group analyses those studies which used animal proteins other than casein (e.g. whey and total milk protein) as control. The Panel notes the methodological limitations of this meta-analysis and considers that no conclusions can be drawn from this meta-analysis for the scientific substantiation of the claim.

All of the individual studies described below were either provided in the three meta-analyses described above or provided separately in the consolidated list.

In a double-blind, randomised, controlled parallel trial, 202 healthy post-menopausal women (60 to 75 years) with mean baseline LDL-cholesterol concentrations of 4.1 mmol/L were assigned to receive daily 25.6 g soy protein containing 99 mg isoflavones (n=100) or total milk protein (n=102) as a powder for 12 months (Kreijkamp-Kaspers et al., 2004). Power calculations were made for three outcome variables, including total cholesterol. It was estimated that 100 subjects per group had to be recruited to detect a 7.4 % difference in total cholesterol concentrations between groups with a power of 80 %, α=0.05, and a drop-out rate of 25 %. A total of 49 subjects withdrew from the study (24 in the placebo group, 25 in the soy group). A modified intention-to-treat analysis was used including all subjects with at least two measurements, including baseline plus a close-out visit (n=22, 9 in the placebo group (n=87 analysed), 13 in the soy group (n=88 analysed)). No statistically significant differences were found in total or LDL-cholesterol concentrations between the soy and the placebo group. The Panel notes that this adequately powered study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised, controlled parallel intervention 213 healthy subjects (105 women, 50-75 years) with mean baseline blood LDL-cholesterol concentrations of approx. 3.85 mmol/L were assigned to consume daily either 40 g of soy protein powder with 188 mg of soy isoflavones (n=105) or casein powder (n=108) to be mixed with beverages for three months (Teede et al., 2001). Thirty-four subjects (19 in the soy group and 15 in the placebo group did not complete the study and were not taken into account in the analysis (n=86 analysed on the soy group and n=93 analysed in the placebo group). No statistically significant differences were observed between groups with respect to total or LDL-cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

The randomised, double-blind, controlled parallel study by Allen et al. (2007) was designed to assess the effects of soy isoflavones in soy protein on LDL-cholesterol concentrations. A total of 216 post-menopausal women (mean age: approx. 57 years) with mean baseline LDL-cholesterol concentrations of approx. 3.6 mmol/L were assigned to consume 20 g/day soy protein containing 160 mg total isoflavones (96 mg aglycones; n=107) or 20 g/day whole milk protein (control; n=109) for 12 weeks after a one-month, single-blind, run-in period to select women with high (>80 %) compliance with the study products. A total of 25 women (14 in the soy and 11 in the control group) dropped out during the study and their baseline measures were used for missing data. The authors reported a statistically significant decrease in total and LDL-cholesterol concentrations after controlling for associated variables (age, race, weight change, change in dietary fat intake, energy expenditure) in the soy compared to the placebo group at week 6 but not at week 12. The Panel notes that carrying forward observations is not an appropriate method of taking into account missing data and that this study does not show a sustained effect of soy isoflavones in soy protein on blood cholesterol concentrations.

A randomised, double-blind, controlled parallel trial which included 182 hyperlipidaemic men and women (44 % male, 30-70 years, mean baseline cholesterol concentrations not reported), assessed the effects on blood cholesterol concentrations of the consumption of a supplement containing 31.5 g/day soy protein and 120 mg/day isoflavones (as aglycones) (n=81 analysed) compared to a milk protein supplement (28.5 g/day; n=78 analysed) for five weeks after a three-week run-in period on the milk protein supplement (Ma et al., 2005). Twenty-two subjects dropped out (six before randomisation and eight in each group during the study), and a further subject was not included in the analysis, which was performed in completers only. No statistically significant differences were found between the two groups with respect to changes in total or LDL-cholesterol concentrations. The Panel notes that missing data were taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised cross-over study 43 male subjects (mean age: approx. 28 years) with mean baseline blood LDL-cholesterol concentrations of 2.74±1.06 mmol/L consumed daily either ISP containing 0.75 mg/kg body weight of isoflavones (around 32 g ISP with 62 mg isoflavones per day) (ISP+), ISP containing low amounts of isoflavones (around 1.6 mg per day) (ISP-) and milk protein powder for periods of 57 days each with a 28-day wash-out period in between (McVeigh et al., 2006). Four subjects dropped out during the study and a further four were excluded from analysis (35 subjects analysed). No statistically significant differences were observed between any of the periods with respect to total or LDL-cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

The double-blind, randomised, controlled parallel intervention by Vigna et al (2000) assessed the effects on cholesterol concentrations of daily consumption of 60 g ISP containing 76 mg isoflavones (n=51) compared to casein (n=53) in 104 post-menopausal women (mean age: approx. 53 years) with mean baseline LDL-cholesterol concentrations of approx. 4.2 mmol/L. Eleven subjects from the ISP group and 14 subjects from the placebo group dropped out during the study, and a further two subjects from the placebo group were excluded from analysis (n=40 analysed in the ISP group and n=37 analysed in the placebo group). No statistically significant differences were found between the two groups with respect to changes in total or LDL-cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised cross-over study, 42 post-menopausal women (mean age: approx. 55 years) with mean baseline blood LDL-cholesterol concentrations of 2.89±0.1 mmol/L consumed ISP with 107.67 mg of total isoflavones (aglycone units), ethanol-washed ISP with 1.82 mg total isoflavones, and total milk protein, in a daily dose of 25 g protein for periods of six weeks each with a four-week wash-out period in between (Steinberg et al., 2003). A total of 14 women withdrew from the study and were not taken into account in the analysis (28 subjects analysed). There were no statistically significant changes in total or LDL-cholesterol concentrations between any of the periods. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised, controlled parallel intervention, 69 women (42-62 years) (baseline total or LDL-cholesterol concentrations not reported) received either ISP containing 80.4 mg/day isoflavone aglycone components (ISP+, n=24), 4.4 mg/day isoflavone aglycone components (ISP-, n=24) or control (whey protein, n=20) in the form of muffins and soy or placebo powder as meal replacements for 24 weeks in four different cohorts in which the interventions were equally represented (Dent et al., 2001). One control subject was not included in the analyses. No statistically significant effect of soy isoflavones on total or LDL-cholesterol concentrations between groups was found at any time point (12 and 24 weeks). The Panel notes that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

The paper by Mackey et al. (2000) reported on two human intervention studies, one of which was a sequential study without wash-out period in between and for which it was unclear whether the control diet was matched to the intervention diet with respect to macronutrient composition, which could have an impact on the claimed effect. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim. The other study reported in the paper was a double-blind, randomised, controlled parallel intervention in 54 post-menopausal women (mean age: approx. 56 years) with total cholesterol concentrations >5.5 mmol/L and mean baseline LDL-cholesterol concentrations of 5.1 mmol/L, who consumed daily 28 mg of soy protein powder containing 65 mg isoflavones (ISP[+], n=22 analysed) or <4 mg isoflavones (ISP[-], n=24 analysed) after a four-week run-in period in which the dietary guidelines from the National Heart Foundation were followed. Eight subjects did not complete the study (group not reported) and were not taken into account in the analysis. No statistically significant differences were observed between groups with respect to total or LDL-cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a randomised, single-blind, controlled parallel trial, 43 obese women (20-65 years) with mean baseline LDL-cholesterol concentrations of approx. 3.0 mmol/L were assigned to consume daily, in the context of an energy-restricted diet, either three soy-based isoflavone-containing (91 g protein, 150 mg isoflavones (aglycones); n=22) or three casein-based (n=21) meal replacement shakes for 16 weeks after a two-week run-in period (Anderson et al., 2007). The shakes were matched with respect to their macronutrient composition, but differed in their calcium, potassium and isoflavone content. Eight women did not complete the study (three in the casein and five in the soy group) and were not taken into account in the analysis (n=17 analysed in the soy group and n=18 analysed in the placebo group). No statistically significant differences were observed between the soy and the casein group with respect to total or LDL-cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised cross-over trial Merz-Demlow et al. (2000) studied the effects of consuming 75-100 g of soy powder (depending on the subject's body weight), which provided an average of 53 g protein and 10 mg (control), 65 mg (low isoflavone) and 129 mg (high isoflavone) soy isoflavones (aglycone units), on cholesterol concentrations in the different phases of the menstrual cycle in 20 women (mean age: 26.3±4.8 years). The intervention covered three menstrual cycles plus nine days, with wash-out periods of 2-3 weeks in between during which an *ad libitum* diet was consumed. Six subjects did not complete the intervention and a further subject was excluded from analysis (13 subjects analysed). Compared with the control diet the high isoflavone diet lowered LDL-cholesterol concentrations significantly, by 7.6 % and 10 %, in the midfollicular and periovulatory phases, respectively, (p<0.02) but not in the early follicular and midluteal phases. The Panel notes that this study evaluated cholesterol lowering effects during menstrual phases only, and did not show a sustained effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised, controlled parallel trial, 57 post-menopausal women (47-72 years) with mean baseline LDL-cholesterol concentrations of approx. 3.7 mmol/L were allocated to consume 40 g ISP containing either 1.2 mg isoflavones and 0.22 g phytate (LP/LI, n=14 analysed), 1.2 mg isofavones and 0.64 g phytate (NP/LI, n=13 analysed), 85.8 mg isoflavones and 0.22 g phytate (LP/NI, n=14 analysed), or 84.6 mg isoflavones and 0.78 g phytate (NP/NI, n=14 analysed) in the form of smoothies or other foodstuffs for six weeks (Engelman et al., 2005). Two subjects did not complete the study (group not reported) and were not taken into account in the analysis. No statistically significant differences were observed between groups with respect to total or LDL-cholesterol concentrations. The Panel notes that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a 15-month, double-blind, randomised, controlled parallel intervention, which involved nine months of dietary intervention and a six-month (intervention-free) follow-up, 65 post-menopausal women (mean age: approx. 55 years) with mean baseline cholesterol concentrations of approx. 3.5 mmol/L were assigned to consume daily either 40 g ISP with 96 mg isoflavones (n=17 analysed), 40 g ISP with 52 mg isoflavones (n=19 analysed) or ISP containing 4 mg isoflavones (n=14 analysed) (Gallagher et al., 2004). Fifteen women (group not reported) dropped out during the study and were not taken into account in the analysis. No statistically significant differences were observed between groups with respect to total or LDL-cholesterol concentrations at the end of the intervention. The Panel notes that missing data were not taken into account in the analysis, and that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised cross-over trial, Meinertz et al. (2002) studied the effect of consuming total liquid diets composed of casein, soy protein or ethanol-extracted soy protein in 12 subjects (6 women; 22-68 years) with mean baseline LDL-cholesterol concentrations of 2.1±0.5 mmol/L for 32 days each, with wash-out periods of at least 18 days in between in which self-selected solid foods were consumed. The Panel assumes that the diets provided 140 g of ISP with 335 mg isoflavones, 140 g of ISP with 15.4 mg of isoflavones and 139 g of casein. No information on drop-outs was provided. No statistically significant differences were observed between the three diets with respect to total or LDL-cholesterol concentrations. The Panel notes that this study does not show an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

The double-blind, controlled parallel intervention by Gardner et al. (2001) assessed the effect of daily consumption during 12 weeks of 42 mg ISP containing 80 mg of isoflavones (ISP 80; n=31 analysed) or trace amounts of isoflavones (ISP$^-$, n=33 analysed), or milk protein (n=30 analysed) to be mixed with beverages in 115 post-menopausal women (mean age: approx. 60 years) with mean baseline LDL-cholesterol concentrations of approx. 4.2 mmol/L. During the four-week run-in phase in which the milk protein supplement was consumed, and before randomisation, 15 women dropped out, and a further six (three in the ISP 80, one in the ISP$^-$ and two in the milk protein group) did not complete the study after randomisation and were not taken into account in the analysis. It was estimated that 30 subjects per group would provide 80 % power to detect a difference in change in LDL-cholesterol concentrations of 10 %, using an α=0.05. A statistically significant difference between the ISP 80 and the ISP$^-$ group was observed with respect to total and LDL-cholesterol concentrations at the end of the study (-0.27 *vs*. -0.02 mmol/L, p=0.03 and -0.38 *vs*. -0.09 mmol/L, p=0.005, respectively). No significant differences were observed between any of the ISP groups compared to the milk protein group. The Panel notes that missing data were not taken into account in the analysis and that this study shows an effect on blood cholesterol concentrations of ISP$^+$ when compared to ISP$^-$, but not if ISP$^+$ was compared to animal protein. The Panel also notes that the results of this study are inconsistent with respect to the effects of soy isoflavones in soy protein on blood cholesterol concentrations.

In a randomised, cross-over study (Ashton and Ball, 2000), 45 healthy males (34-62 years) with mean baseline LDL-cholesterol concentrations of 3.68±0.86 mmol/L consumed a tofu-based diet (290 g tofu/day) providing 80 mg isoflavones per day, and a meat-based diet (150 g cooked lean red meat), for four weeks each with a two-week wash-out period in between. All subjects consumed similar vegetarian breakfasts, lunches and snacks on both diets, and diets were comparable with respect to macronutrient composition (except for the amount of cholesterol they provided). Three subjects did not complete the study and were not taken into account in the analysis (42 subjects analysed). Compared to the meat diet, total cholesterol concentrations were significantly lower in the tofu diet period (mean difference: 0.23, 95 % CI 0.02, 0.43), whereas no statistically significant differences were observed for LDL-cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones in soy foods on total blood cholesterol concentrations.

In a randomised, blinded, controlled parallel intervention, 82 post-menopausal women (aged 45-55 years) with mean baseline LDL-cholesterol concentrations of 3.4 mmol/L were assigned to consume daily either 150 mg soy protein and 100 mg isoflavones in capsules (n=41) or placebo capsules containing the same amount of isoflavone-free soy protein plus glucose (n=41) for four months (Han et al., 2002). One subject from each group dropped out during the study. Total and LDL-cholesterol concentrations significantly decreased in the isoflavone group compared to placebo (mean change: -26.6±1.2 mg/dL *vs.* 0.2±0.4 mg/dL, and -13.3±1 mg/dL *vs.* 5.5±1.2 mg/dL, for total and LDL–cholesterol concentrations, respectively, p<0.001). The Panel notes that only basic statistical analyses were performed, and that this study shows an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, placebo controlled, parallel trial, 159 subjects (25 women; mean age: 52 years) with LDL-cholesterol concentrations ≥4 mmol/L, after having followed an American Heart Association Step I diet for 4-24 weeks and having mean baseline LDL-cholesterol concentrations of approx. 4.8 mmol/L, were randomised to one of four groups to consume in the form of beverages, while continuing the Step I diet: 30 g ISP with 111 mg isoflavones and 10 g cotyledon fibre (n=39): 50 g ISP with 185 mg isoflavones and 16.6 g cotyledon fibre (n=40): 30 g casein and 10 g cellulose (n=40) or 50 g casein and 16.6 g cellulose (n=40) for 16 weeks (Tonstad et al., 2002). The study was designed to have 90 % power to detect a 10 % relative change in total cholesterol concentrations (assuming an SD of 10 %) between groups for pair-wise comparisons with a two-sided p<0.05. Nineteen subjects did not complete the study and a further 10 were not included in the per protocol analysis (five in the 30 g ISP group (n=34 analysed), four in the 30 g casein group (n=36 analysed), nine in the 50 g ISP group (n=31 analysed) and 11 in the 50 g casein group (n=29 analysed)). In addition, all subjects who received the intervention for four weeks or longer were included in a modified intention-to-treat analysis. In the per-protocol analysis there was a statistically significant difference in the decrease in total and LDL-cholesterol concentrations between the two ISP and the two casein groups, as pooled together (difference in change: -0.24, 95 % CI -0.43, -0.04 and -0.26, 95 % CI -0.43, -0.09, p=0.01 for the interaction between time and treatment). The modified intention-to-treat analysis yielded similar results. The interaction between time and dose was not significant. The Panel notes that separate analyses for the different groups as compared to the respective controls were not reported. The Panel notes that this study shows an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised, controlled parallel intervention, 156 subjects (62 females; 20-70 years), with baseline LDL-cholesterol concentrations between 3.6 and 5.2 mmol/L after a one-month run-in period on a National Cholesterol Education Program (NCEP) Step I diet, and a further month on casein beverages (in addition to the NCEP Step I diet), were allocated to consume daily one of the following: 25 g of soy protein with either 3 mg isoflavones (ISP 3; n=28 analysed), 27 mg isoflavones (ISP 27; n=27 analysed), 37 mg isoflavones (ISP 37; n=30 analysed) and 62 mg isoflavones (ISP 62; n=30 analysed) or 25 g casein (n=31 analysed) for nine weeks (Crouse et al., 1999). The study was designed to enrol 30 participants per group to evaluate 25 (assuming drop-outs) and have 95 % power to detect a 6 % relative change in LDL-cholesterol concentrations between groups for pair-wise group comparisons at the 5 % two-sided level of significance. Twelve subjects (three in the casein, four in the ISP 3 and five in the ISP 27 group) did not complete the study. Plasma lipids were measured at weeks 8 and 9, and an average of these two measurements was taken for analysis. The analysis was reported to have been performed on an intention-to-treat basis, although from the information provided it appeared that data from ten subjects were not taken into account in the analysis. Only the ISP 62 group significantly reduced total cholesterol concentrations, i.e. by 4 % (-0.25 mmol/L; 95 % CI -0.49, -0.0003), and LDL-cholesterol concentrations, i.e. by 6 % (-0.27 mmol/L; 95 % CI -0.47, -0.06) as compared to casein. There was a statistically significant dose-response relationship between the intake of isoflavones and a decrease in total and LDL-cholesterol concentrations (p=0.01 and p=0.02 for the trends for total and for LDL-cholesterol concentrations, respectively). No effect on either total or LDL-cholesterol concentrations was

observed for ISP 3 or ISP 27. The Panel notes that this study shows an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

In a double-blind, randomised controlled parallel trial, 81 post-menopausal women (mean age: approx. 60 years) with baseline plasma cholesterol concentrations between 6.2 and 7.8 mmol/L and mean baseline blood non-HDL-cholesterol concentrations of approx. 1.4 mmol/L, after having followed a 2-week run-in period on an NCEP Step I diet, were studied during three separate 24-week cohorts in which subjects were assigned to consume daily, in the context of an NCEP Step I diet, 40 g ISP containing 56 mg aglycone isoflavones (ISP 56; n=23 analysed), 40 g ISP containing 90 mg aglycone isoflavones (ISP 90; n=21 analysed) or 40 g casein and non-fat dry milk in the form of baked goods or ready-to-mix beverages and soups (Baum et al., 1998). Seven subjects dropped out during the intervention, and a further eight were not taken into account in the analysis (group not reported). No statistically significant differences were observed with respect to total cholesterol concentrations for either of the two ISP groups compared to placebo at any time point. Non-HDL cholesterol concentrations were significantly different between the ISP 56, the ISP 90 and the control groups (adjusted (for baseline means) mean difference: -0.28 and -0.25 mmol/L; p=0.03 and p=0.04, respectively) at the end of the study. The Panel notes that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones in soy protein on blood non-HDL cholesterol concentrations.

In a double-blind, randomised cross-over study, 23 post-menopausal women (mean age: approx. 57 years) with mean baseline blood LDL-cholesterol concentrations of approx. 3.5 mmol/L consumed daily 63 g ISP with 7.1±1.1 mg isoflavones (control diet), 65±11 mg isoflavones (low isoflavone diet), and 13±22 mg isoflavones (high isoflavone diet) for periods of 93 days each with a 26-day wash-out period in between (Wangen et al., 2001). Four subjects dropped out during the first diet period, and a further one was excluded from the analysis (18 subjects were analysed for the control and high isoflavone diet and 17 for the low isoflavone diet). Repeated measures analysis of variance showed a statistically significant difference between the three diets with respect to LDL-cholesterol concentrations (p=0.01), but not with respect to total cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones in soy protein on blood LDL-cholesterol concentrations.

In a double-blind, randomised cross-over trial, Hermansen et al. (2001) studied the effect on blood cholesterol concentrations of consuming 50 g ISP with >165 mg soy isoflavones and 20 g soy cotyledon fibre as compared to 50 g casein and 20 g cellulose for six weeks with a three-week wash-out period in between in 25 type 2 diabetic subjects of whom 20 completed the intervention (6 women, mean age approx. 64 years, mean baseline LDL-cholesterol concentrations: approx. 3.6 mmol/L). Analysis was performed in the population of completers. The percentage mean treatment difference between the two periods was statistically significant for LDL-cholesterol concentrations (10±15 %, p<0.05), but not for total cholesterol concentrations. The Panel notes that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones in soy protein on blood LDL-cholesterol concentrations.

In a double-blind, randomised, controlled parallel intervention, 92 men (23-74 years) with baseline total cholesterol concentrations between 5.69 and 7.76 mmol/L were allocated, after a three-week run-in period on an NCEP Step I diet, to consume daily either 50 g ISP with 95 mg isoflavones (n=15 analysed), 40 g ISP, 10 g casein and 76 mg isoflavones (n=17 analysed), 30 g ISP, 20 g casein and 57 mg isoflavones (n=18 analysed), 20 g ISP, 30 g casein and 38 mg isoflavones (n=15 analysed) or 50 g casein (n=16 analysed) in the form of baked goods or ready-to-mix beverages for six weeks in three separate cohorts (Teixeira et al., 2000). Eight subjects dropped out during the study, and a further three were not taken into account in the analysis (groups not reported). Non-HDL cholesterol concentrations were significantly different in all ISP groups compared to control at six weeks (adjusted (for baseline) mean change: -0.182 (50 g ISP), -0.095 (40 g ISP), -0.163 (30 g ISP),

-0.139 (20 g ISP) *vs*. +0.224 (control) mmol/L, p<0.05). Total cholesterol changes were significant for the 50 g, 30 g and 20 g ISP groups (but not for the 40 g ISP group) as compared to control at six weeks (adjusted mean change: -0.167 (50 g ISP), -0.115 (30 g ISP), -0.126 (20 g ISP) *vs*. 0.222 (control) mmol/L, p<0.05). The Panel notes that missing data were not taken into account in the analysis and that this study shows an effect of soy isoflavones in soy protein on blood cholesterol concentrations.

The Panel notes that out of the 23 human intervention studies in which soy isoflavones were consumed in soy protein from which conclusions could be drawn for the scientific substantiation of the claim, 14 studies (n=1,286 subjects, 12-93 subjects per group/period) with durations between one and 12 months using isoflavone doses of around 60-330 mg per day did not show an effect of soy isoflavones on blood cholesterol concentrations in subjects with slightly elevated or high blood LDL-cholesterol concentrations, whereas eight studies (n=712 subjects, 15-42 subjects per group/period) with durations between six weeks and six months using isoflavone doses of around 30-185 mg per day showed a statistically significant effect in subjects with normal to high blood LDL-cholesterol concentrations, and one study (n=94 subjects, around 30 per group) led to inconsistent results with respect to the effect of soy isoflavones on blood LDL-cholesterol concentrations. The Panel also notes that most of the studies had some methodological limitations, and that the inconsistent results reported appear unrelated to the dose of isoflavones used, the study duration, the sample size or the baseline characteristics of subjects with respect to blood cholesterol concentrations.

In weighing the evidence, the Panel took into account that one meta-analysis of nine RCTs and three additional RCTs did not show an effect of extracted soy isoflavones on blood cholesterol concentrations and that the evidence provided by 23 RCTs in which soy isoflavones were consumed in soy protein is inconsistent.

The Panel concludes that a cause and effect relationship has not been established between the consumption of soy isoflavones and maintenance of normal blood LDL-cholesterol concentrations.

3.3. Reduction of vasomotor symptoms associated with menopause (ID 1654, 1704b, 2140, 3093b, 3154, 3590)

Among the references provided for the scientific substantiation of the claim were narrative reviews which were either unrelated to the claimed effect or did not contain any original data which could be used for the scientific substantiation of the claim. A number of human, animal and *in vitro* studies were unrelated to the claimed effect. These references included studies on the effect of soy isoflavones on blood lipid concentrations, severity of asthma, DNA damage, mood, cognitive function, bone loss, bone fractures, cancer incidence, LDL peroxidation, weight loss, inflammation, cardiovascular disease risk and endothelial function. Some human intervention studies used food constituents other than soy isoflavones (e.g. red clover isoflavones) or a combination of soy isoflavones with other constituents (Brzezinski et al., 1997; Campagnoli et al., 2005). Three publications were available only in the form of a brief communication (Jou et al., 2005; Ricciotti et al., 2005) or an abstract (Imhof et al., 2008) which did not provide sufficient information with regard to the study design and the statistical analysis for a full scientific evaluation. One prospective cohort study (Nagata et al., 2001) and two cross-sectional studies (Nagata et al., 1999; Somekawa et al., 2001) which investigated the association of consumption of soy isoflavones and soy products with vasomotor symptoms associated with menopause were provided. The Panel notes that these studies were not sufficiently controlled for other dietary factors or medication use both of which could have an effect on vasomotor symptoms. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

Two meta-analyses (Howes et al., 2006; Nelson et al., 2006), six systematic reviews (Howes et al., 2006; Huntley and Ernst, 2004; Kronenberg and Fugh-Berman, 2002; Lethaby et al., 2007; Nelson et al., 2006; Tempfer et al., 2007) and 39 human intervention studies which addressed the effect of soy isoflavone consumption on vasomotor symptoms associated with menopause were provided either separately in the consolidated list or in the systematic reviews/meta-analyses provided.

Two of the studies provided were open-label, one-arm, uncontrolled human interventions in 190 (Albert et al., 2002) and 169 (Drews et al., 2007) post-menopausal women, respectively. The Panel considers that no conclusions can be drawn from these uncontrolled studies for the scientific substantiation of the claim. Some RCTs included women with a diagnosis of breast cancer and/or on tamoxifen therapy (MacGregor et al., 2005; Nikander et al., 2003; Petri Nahas et al., 2004; Quella et al., 2000; Secreto et al., 2004; Van Patten et al., 2002). In one study, tamoxifen use was not an exclusion criterion, and the number of women recruited who were on tamoxifen (if any) was not reported (Albertazzi et al., 1998). The Panel notes that no evidence has been provided that women with breast cancer and/or on tamoxifen therapy are representative of the target population with respect to the frequency and/or severity of hot flushes and night sweats, or that results obtained in women with breast cancer and/or on tamoxifen therapy can be extrapolated to menopausal women in the general population. In one study, hormone replacement therapy (HRT), which could have an impact on the claimed effect, was started by some of the subjects during the study (Burke et al., 2003) and in one study (Penotti et al., 2003) the use of HRT or other medication was not reported to be an exclusion criterion, and the number of women (if any) recruited who were on HRT or on other medication which could have an effect on vasomotor symptoms was not reported. In another study (Lewis et al., 2006), some of the subjects took, throughout the study, medication such as *Cimicifuga racemosa* (L.) Nutt. or therapy for thyroid disease, which could have had an impact on the claimed effect, or during the study started antibiotic therapy which had been defined as an exclusion criterion for the study. In three of the studies (Colacurci et al., 2004; Washburn et al., 1999; Welty et al., 2007a) subjects were not appropriately blinded to the intervention (i.e. the effects of soy isoflavones in various forms were compared to no treatment, or the control and intervention were not consumed at the same daily frequency). The Panel notes that successful blinding of subjects is particularly important to evaluate self-reported health outcomes, such as frequency and severity of hot flushes, for which a high placebo effect can be expected. The description of the methods used for statistical analyses was not provided in two studies (Caserta et al., 2005; Uesugi et al., 2004), one study did not report on the amount of isoflavones used (Murkies et al., 1995), two studies did not report on vasomotor symptoms specifically but on scores for menopausal symptoms combined using the Kupperman Index (Sammartino et al., 2003), or on general quality of life questionnaires (Kok et al., 2005), and one study used the Menopause-Specific Quality of Life Questionnaire, which only provides information about the occurrence and degree of bother caused by menopausal symptoms, including vasomotor symptoms, but not on their frequency or severity (Basaria et al., 2009). In one cross-over study only within-treatment, but no between-treatment, comparisons were reported (Dalais et al., 1998), in one study randomisation did not take into account the pre-planned sub-group analysis of equol and non-equol producing subjects, and no overall results were reported (Jou et al., 2008), and in a further study in which the presence of menopausal symptoms was not an inclusion criterion, no information was provided on whether subjects were comparable at baseline with regard to menopausal symptoms, and whether the use of HRT or other medication, which could have had an impact on the claimed effect, was an exclusion criterion for the study (Scambia et al., 2000). In two small sample size studies (Balk et al., 2002; Duffy et al., 2003) the presence of menopausal symptoms was not an inclusion criterion, and the sample size of women experiencing menopausal symptoms in these studies might have been insufficient to detect a statistically significant difference between the intervention and the control, and in one study (Kaari et al., 2006) the effect of soy isoflavones was compared to oestrogen therapy but lacked a negative control. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

In the meta-analysis by Howes et al. (2006), four electronic databases were searched (time span of search not reported) for parallel design RCTs of at least four weeks which compared an isoflavone intervention to a non-isoflavone, non-oestrogenic control, and reported the frequency and variance of hot flushes. Twelve studies on soy isoflavones met the inclusion criteria (Albertazzi et al., 1998; Burke et al., 2003; Colacurci et al., 2004; Faure et al., 2002; Han et al., 2002; Knight et al., 2001; Murkies et al., 1995; Penotti et al., 2003; Scambia et al., 2000; St Germain et al., 2001; Upmalis et al., 2000; Van Patten et al., 2002). The Panel notes that study quality was not assessed in this meta-analysis and that some of the studies included did not allow conclusions to be drawn on the effects of isoflavones on the frequency of hot flushes owing to the following limitations: inclusion of women with breast cancer on tamoxifen (Van Patten et al., 2002), uncertainty as to whether women were on tamoxifen (Albertazzi et al., 1998), uncertainty as to whether subjects were blinded to the intervention (Colacurci et al., 2004), uncertainty on the amount of isoflavones provided (Murkies et al., 1995), and uncertainty as to comparability at baseline in the study groups (Scambia et al., 2000). The Panel considers that no conclusions can be drawn from these studies, and thus from the meta-analysis for the scientific substantiation of the claim.

In the meta-analysis by Nelson et al. (2006), several databases were searched up to October 2005 for RCTs in English on the effect of non-hormonal therapies on the frequency and severity of hot flushes. In addition, systematic reviews were hand searched. Eleven trials on soy isoflavone extracts met the inclusion criteria. Trials were included if they measured frequency or severity of hot flushes. Six (Crisafulli et al., 2004; Faure et al., 2002; Penotti et al., 2003; Quella et al., 2000; Scambia et al., 2000; Upmalis et al., 2000) out of the 11 trials provided data for a meta-analysis. The Panel notes that this meta-analysis included a study in patients with breast cancer on tamoxifen medication (Quella et al., 2000), as well as a trial considered by the authors as being of poor quality (Scambia et al., 2000). Results were reported overall and excluding either the study in breast cancer patients or the poor quality trial, but not excluding both. The Panel considers that no conclusions can be drawn from this meta-analysis for the scientific substantiation of the claim.

From the remaining human intervention studies provided, either separately in the consolidated list or in the systematic reviews, seven investigated the effect of soy isoflavones on both frequency and severity of hot flushes (Albertazzi et al., 2005; D'Anna et al., 2007; Khaodhiar et al., 2008; Knight et al., 2001; Nahas et al., 2007; St Germain et al., 2001; Upmalis et al., 2000), two investigated frequency only (Crisafulli et al., 2004; Faure et al., 2002) and three severity only (Cheng et al., 2007; Han et al., 2002; Kotsopoulos et al., 2000). Three of these studies also investigated the effect of soy isoflavones on night sweats (Cheng et al., 2007; St Germain et al., 2001; Upmalis et al., 2000). In all of these studies, subjects in the intervention and control groups within these studies were not different at baseline with regard to the frequency and/or severity of hot flushes and/or night sweats.

In eight out of nine studies which investigated the effect of soy isoflavones on the frequency of hot flushes, frequency of hot flushes was self-assessed in symptom diaries or cards, in which the daily symptoms were noted, while in the remaining study by St Germain et al. (2001) this outcome was assessed by an interviewer-administered menopausal index. In the 10 studies which investigated the effect of soy isoflavones on severity of hot flushes, severity of hot flushes was assessed by self-rating scales in five studies (Cheng et al., 2007; D'Anna et al., 2007; Khaodhiar et al., 2008; Nahas et al., 2007; Upmalis et al., 2000), by the vasomotor sub-scale of the Greene Climacteric Scale in two studies (Albertazzi et al., 2005; Knight et al., 2001), by the vasomotor symptom score of the Kupperman Index in one study (Han et al., 2002), by an interviewer-administered menopausal index in one study (St Germain et al., 2001), and by a validated questionnaire in the remaining study (Kotsopoulos et al., 2000). In the three of the aforementioned studies which investigated night sweats, one study (Cheng et al., 2007) assessed the severity of night sweats only, one study (Upmalis et al., 2000) assessed the frequency of night sweats only, while St Germain et al. (2001) assessed both of these measures of night sweats.

In the double-blind, randomised cross-over study by Albertazzi et al. (2005), 100 post-menopausal women (44-65 years) were allocated to consume daily 90 mg genistein and placebo for six weeks each without a wash-out period in between; the cross-over design had not been pre-planned. Subjects had a mean hot flush score (frequency x severity) of 7 at baseline, but the study also included subjects who were not experiencing any symptoms. The primary endpoint of the study was markers of bone turnover. One subject dropped out in the placebo group. Statistical analysis was adjusted for sequence and time to exclude a carry-over and a period effect. Frequency and severity of hot flushes were not significantly different between the genistein and the placebo periods. Subjects with a hot flush score >9 were analysed in a *post-hoc* analysis (n=41). The Panel notes that this sub-group analysis was not pre-planned, and considers that no conclusions can be drawn from the sub-group analysis for the scientific substantiation of the claim. The Panel also notes that not all subjects included in the study were experiencing vasomotor symptoms, and that the overall results of this study do not show an effect of genistein on the frequency or severity of hot flushes.

In the double-blind, randomised, placebo-controlled study by Khaodhiar et al. (2008) 191 post-menopausal women (aged 38-60 years) experiencing at least four hot flushes (mean frequency: approx. 8, mean severity approx. 2.1 on a 4-point scale) per day were randomised to consume daily either 40 mg daidzein-rich isoflavone aglycones (DRI) (n=48 analysed), 60 mg DRI (n=49 analysed) or placebo (n=45 analysed) in capsule form for 13 weeks. It was calculated that 50 women per group would be needed to detect with an 80 % power an average difference between groups of 1.2 hot flushes per day and a hot flush score (frequency x severity) of three units per day with a 0.58 SD and a p-value <0.05. Forty-nine subjects did not complete the study (group not reported) and were not taken into account in the analysis. Hot flush frequency and severity was not significantly different between the 40 mg or 60 mg DRI groups compared to placebo at any time point (4, 8 and 12 weeks). In a *post-hoc* analysis, data from week 12 from both DRI groups were pooled together and compared to placebo. The Panel notes that the data pooling was not pre-planned, and considers that no conclusions can be drawn from this *post-hoc* analysis for the scientific substantiation of the claim. The Panel also notes the high drop-out rate, that missing data were not taken into account in the analysis, and that the primary analysis of this study does not show an effect of daidzein-rich isoflavone aglycones on the frequency or severity of hot flushes.

In the double-blind, randomised, placebo-controlled trial by Kotsopoulos et al. (2000), 94 post-menopausal women (mean age 59.5 years) of whom 80 % were experiencing menopausal symptoms (not different between groups, mean symptom score approx. 0.8 on a 3-point scale) were randomised to consume either 118 mg isoflavones in soy powder (n=44) or casein powder (n=50) twice daily in sachet form taken in the form of beverages for 12 weeks. Nineteen subjects withdrew from the study (10 in the soy and 9 in the placebo group), and a further two in the soy group were not taken into account in the analysis (n=32 analysed in the soy group and n=41 analysed in the placebo group). No statistically significant differences were observed between the intervention and control group with respect to vasomotor symptom scores at 12 weeks. No other time points were measured. The Panel notes that missing data were not taken into account in the analysis and that this study does not show an effect of soy isoflavones on the severity of hot flushes.

In a double-blind, randomised, controlled, multicentre study, 75 post-menopausal women (mean age: 53 years) with at least seven hot flushes (including night sweats) per day (mean frequency: 10) during a 2-week pre-study period were allocated to consume daily for 16 weeks either 70 mg soy isoflavones in the form of a soy extract (n=39) or placebo (n=36, microcrystalline cellulose and magnesium stearate) (Faure et al., 2002). A sample size of 30 subjects per arm was required to detect, with 90 % power, a difference of three hot flushes per 24 hours, assuming an SD of 3.8 with a p<0.05. The primary endpoint of the study was the number of daily moderate to severe hot flushes (including night sweats) in each month of treatment. Six subjects dropped out in the soy group, and 14 in the placebo group. Data analysis was reported to have been performed per protocol, and as "last observation carried forward". The Panel notes that carrying forward the last observation is not an appropriate

method of taking into account missing data. Per protocol/completer analysis did not show a statistically significant difference between the soy and placebo groups in numbers of moderate to severe hot flushes. The Panel notes the high drop-out rate, and that the primary analysis of this study does not show an effect of soy isoflavones on the frequency of hot flushes.

In the double-blind, placebo-controlled intervention by St Germain et al. (2001), 69 peri-menopausal women (aged 42-62 years) who were experiencing at least 10 vasomotor symptoms per week (mean frequency and severity not reported) received either isolated soy protein containing 80.4 mg/day isoflavone aglycone components (ISP⁺, n=24), 4.4 mg/day isoflavone aglycone components (ISP⁻, n=24) or control (whey protein, n=21) in the form of muffins and soy or placebo powder as meal replacements for 24 weeks in four different cohorts in which the interventions were equally represented. One control subject was not included in the analysis. No statistically significant effect of soy isoflavones on the reduction of frequency or severity of hot flushes and night sweats between groups was found at any time point (12 and 24 weeks). The Panel notes the lack of power calculations, and that this study does not show an effect of soy isoflavones on the frequency or severity of hot flushes or night sweats.

In the double-blind, randomised, placebo-controlled trial by Knight et al. (2001), 24 post-menopausal women (40-65 years) with at least three hot flushes (mean frequency: approx. 7.5, mean severity not reported) per day were randomised to receive either 134.4 mg soy isoflavones (containing 77.4 mg of aglycones) in 60 g soy powder (n=12) or casein powder (n=12) once daily in sachet form (to be consumed as beverages) for 12 weeks. Three women in the intervention group and one in the placebo group withdrew from the study. Analyses were reported to have been performed in the intention-to-treat population. No statistically significant differences were found between the soy and the placebo groups with regard to frequency and severity of hot flushes at 12 weeks. No other time points were measured. The Panel notes that no power calculations were performed, and that this study does not show an effect of soy isoflavones on the frequency or severity of hot flushes.

In the double-blind, randomised, controlled, multicentre (15 centres) trial by Upmalis et al. (2000), 177 post-menopausal women with at least five hot flushes per day (mean frequency: 9, mean severity: 2 on a 3-point scale) were randomised to receive daily either 50 mg of soy isoflavones in a soy extract in tablet form (n=89) or placebo (not specified, n=86) for 12 weeks. The primary endpoint for efficacy was reported to be the reduction of frequency and severity of hot flushes. Data from 59 subjects in the intervention group and 63 in the placebo group were evaluated for hot flushes and night sweats. There was a statistically significant reduction in severity of hot flushes per week between the soy and the placebo group at week 12 (approx. -27 % *vs*. -19 %, p=0.01). There was a statistically significant reduction in the frequency of hot flushes and episodes of night sweats (as assessed by awakenings during the night) between the soy and the placebo group at week six (approx. -27 % *vs*. -12 %, p=0.02 for hot flushes; approx. -60 % *vs*. -25 % p<0.04 for night sweats), while this difference was not significant at week 12. The Panel notes that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones on the severity of hot flushes, but that soy isoflavones did not have a sustained effect on the frequency of hot flushes and episodes of night sweats. The Panel notes that the results of this study are inconsistent with respect to the effect of soy isoflavones in soy protein on vasomotor symptoms.

In a sub-study of a double-blind, randomised, controlled bone loss trial, D'Anna et al. (2007) analysed 247 (of 389) post-menopausal women (mean age: approx. 53 years) experiencing hot flushes (mean frequency: approx. 4, mean severity: approx. 2.3 on a 3-point scale) who consumed either 27 mg per day of genistein (n=125) or placebo (n=122) for 12 months. It was calculated that at least 97 subjects per group were needed to detect, with an 80 % power in two-sided tests, a difference of 20 % between groups with a p<0.05. Ten subjects dropped out in the genistein group and eight in the placebo group, and were not taken into account in the data analysis (n=115 analysed in the genistein group and n=114 analysed in the placebo group). A statistically significant difference was observed between the

genistein and the placebo groups in the frequency of hot flushes per day at 1 month (mean change -1.1 *vs.* 0.2, p<0.001), at 3 months (mean change -1.8 *vs.* 0.5, p<0.001), at 6 months (mean change -2.0 *vs.* 0.5, p<0.001) and at 12 months (mean change -2.5 *vs.* 0; p<0.001), and in severity scores at 1 month (mean change -0.3 *vs.* 0.1, p=0.005), at 3 months (mean change -0.6 *vs.* 0.1, p<0.001), at 6 months (mean change -0.7 *vs.* 0, p<0.001) and at 12 months (mean change -0.9 *vs.* -0.1; p<0.001). The Panel notes that the study was a sub-analysis of a bone study, and that missing data were not taken into account in the analysis. The Panel also notes that this study shows an effect of genistein on the frequency and severity of hot flushes.

In the randomised, placebo-controlled trial by Han et al. (2002), 82 women (aged 45-55 years) who were experiencing hot flushes (mean severity score approx. 2.5 on a 3-point scale) were assigned to consume daily for four months either 150 mg soy protein and 100 mg soy isoflavones (n=41) or 150 mg soy protein without isoflavones (n=41). One subject in each group dropped out during the study. The vasomotor symptom score of the Kupperman Index significantly decreased in the soy isoflavone group compared to the placebo group (mean change -3.1 *vs.* -0.1; p<0.01) at four months. The Panel notes the basic statistical analyses performed, and that this study shows an effect of soy isoflavones on the severity of hot flushes.

In the double-blind, randomised, placebo-controlled study by Nahas et al. (2007), 80 post-menopausal women (mean age approx. 55 years) with at least five hot flushes (mean frequency: approx. 10, mean severity: approx. 9 on a 12-point scale) per day were allocated to consume daily either 100 mg of isoflavones in 250 mg standardised soy extract (n=40) or placebo (lactose; n=40) for ten months. Four subjects dropped out during the study (two in the intervention group and two in the placebo group) and were not taken into account in the analysis (n=38 analysed in the soy group and n=38 analysed in the placebo group). At the end of the study, the mean frequency of hot flushes decreased significantly in the soy group as compared to placebo (mean change -6.5 *vs.* -4.2, p<0.001), as did the hot flush severity scores (mean change -6.5 *vs.* -2.7, p<0.001). Between-group comparisons for other time points (4 and 7 months) were not reported. The Panel notes that the statistical analysis was not appropriate for the study design, that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones on the frequency and severity of hot flushes.

In the double-blind, placebo-controlled study by Crisafulli et al. (2004), 90 post-menopausal women (47-57 years) experiencing a mean of 4.6 hot flushes per day were randomised to receive either HRT (n=30), 54 mg genistein per day (n=30) or placebo (n=30) for 12 months. The primary endpoint of the study was bone loss. Seven subjects withdrew from taking the intervention or placebo (groups and reasons for withdrawal were not reported) but completed the study and were included in the analysis. Compared to placebo the number of hot flushes decreased significantly after 3 months (-22 %, 95 % -38, -6.2, p<0.01), 6 months (-29 %, 95 % CI -45, -13, p<0.001) and 12 months (-24 %, 95 % CI, -43, -5, p<0.01) of genistein consumption. The Panel notes that the description of the statistical methods used is insufficient, and that this study shows an effect of genistein on the frequency of hot flushes.

In the double-blind, randomised placebo-controlled trial by Cheng et al. (2007), 60 post-menopausal women (49-69 years) with hot flushes and night sweats (mean hot flush and night sweat scores approx. 1.4 on a 5-point scale) were assigned to consume daily either 60 mg isoflavones in a fruit-flavoured drink produced from soy beans (n=26 completers) or a fruit-flavoured oat-meal drink (n=25 completers) for 12 weeks. Both vasomotor symptom frequency and severity were assessed, but results were only reported for severity scores. Data analyses were performed in completers only. At week 12, severity scores for hot flushes were statistically significantly different between the soy and the placebo group (mean change -0.7 *vs.* +0.1; p<0.01), while no differences were found in the severity scores for night sweats. No other time points were measured. The Panel notes the absence of a placebo effect, that missing data were not taken into account in the analysis, and that this study shows an effect of soy isoflavones on the severity of hot flushes but not on the severity of night sweats.

The Panel notes that from the 12 human intervention studies provided from which conclusions can be drawn for the scientific substantiation of the claim, most of which have some methodological limitations and/or inadequate reporting, five studies (n=498 subjects analysed; range: 25-115 per group) with a duration of 12 weeks to 12 months using doses from 27 mg genistein to 100 mg of total isoflavones showed a statistically significant effect of soy isoflavones on vasomotor symptoms after one month (D'Anna et al., 2007), three months (Cheng et al., 2007; Crisafulli et al., 2004), four months (Han et al., 2002) and 10 months (Nahas et al., 2007), whereas six studies (n=463 subjects analysed, range: 12-99 per group/period) with a duration of six weeks to six months using doses from 40 mg to 118 mg of total isoflavones did not show a statistically significant effect of soy isoflavones on vasomotor symptoms (Albertazzi et al., 2005; Faure et al., 2002; Khaodhiar et al., 2008; Knight et al., 2001; Kotsopoulos et al., 2000; St Germain et al., 2001), and that one study in 122 analysed subjects using a dose of 50 mg isoflavones for four months led to inconsistent results with regard to the effect soy isoflavones on vasomotor symptoms.

In weighing the evidence, the Panel took into account that the evidence provided by 12 human intervention studies is inconsistent with respect to the reduction of vasomotor symptoms, and that the inconsistent results cannot be explained by dose, sample size, study duration, or baseline frequency or severity of vasomotor symptoms.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of soy isoflavones and reduction of vasomotor symptoms associated with menopause.

3.4. Contribution to normal hair growth (ID 1704a, 4254)

Among the references provided were a number of narrative reviews and a textbook which were either not related to the claimed effect or did not provide any original data which could be used for the scientific substantiation of the claim. Some of the references provided were not related to the food constituent, which is the subject of the claim, or investigated the effect of soy isoflavones in combination with other substances. A number of human, animal and *in vitro* studies were not related to the claimed effect; these studies included references on the effect of soy isoflavones on symptoms associated with the menopause, asthma severity, DNA damage, mood, cognitive function, bone loss, blood lipids and cancer. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

An *in vitro* study assessed the effect of isoflavones on hair growth in cultured human isolated hair follicles. The Panel considers that evidence provided in *in vitro* studies is not sufficient to predict the occurrence of an effect of soy isoflavone consumption on hair growth *in vivo* in humans.

The Panel concludes that a cause and effect relationship has not been established between the consumption of soy isoflavones and contribution to normal hair growth.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, soy isoflavones, which is the subject of the health claims, is sufficiently characterised.

Protection of DNA, proteins and lipids from oxidative damage (ID 1286, 4245)

- The claimed effects are "vascular effects including protection from oxidative damage" and "antioxidant status". The target population is assumed to be the general population. Protection of DNA, proteins and lipids from oxidative damage may be a beneficial physiological effect.

- A cause and effect relationship has not been established between the consumption of soy isoflavones and protection of DNA, proteins and lipids from oxidative damage.

Maintenance of normal blood LDL-cholesterol concentrations (ID 1135, 1704a, 3093a)

- The claimed effects are "cholesterol management / heart health", "menopause/skin and hair health during menopause/cholesterol management" and "act as phyto-estrogens". The target population is assumed to be the general population. Maintenance of normal blood LDL-cholesterol concentrations is a beneficial physiological effect.

- A cause and effect relationship has not been established between the consumption of extracted soy isoflavones and maintenance of normal blood LDL-cholesterol concentrations.

Reduction of vasomotor symptoms associated with menopause (ID 1654, 1704b, 2140, 3093b, 3154, 3590)

- The claimed effects are "menopause", "menopause/skin and hair health during menopause/cholesterol management", "soy contains the phytoestrogens isoflavones that can function as either an estrogen agonist or antagonist", "act as phytoestrogens", "helps to keep healthy thermoregulation during climacterium", and "helps to alleviate the symptoms of menopause". The target population is assumed to be post-menopausal women. In the context of the proposed wordings and the clarifications provided by Member States, it is assumed that the claimed effects refer to the reduction of vasomotor symptoms associated with menopause. Reduction of vasomotor symptoms associated with menopause is a beneficial physiological effect.

- The evidence provided is insufficient to establish a cause and effect relationship between the consumption of soy isoflavones and reduction of vasomotor symptoms associated with menopause.

Maintenance of normal skin tonicity (ID 1704a)

- The claimed effect is "menopause/skin and hair health during menopause/cholesterol management". The target population is assumed to be post-menopausal women. In the context of the proposed wordings and the clarifications provided by Member States, it is assumed that the claimed effect refers to the maintenance of normal skin tonicity. No evidence has been provided on how skin tonicity could be related to skin function.

- The claim does not refer to a function of the body as required by Regulation (EC) No 1924/2006.

Contribution to normal hair growth (ID 1704a, 4254)

- The claimed effect is "menopause/skin and hair health during menopause/cholesterol management" and "hair growth and loss". The target population is assumed to be the general population. Contribution to normal hair growth is a beneficial physiological effect.

- A cause and effect relationship has not been established between the consumption of soy isoflavones and contribution to normal hair growth.

"Cardiovascular health" (ID 3587)

- The claimed effect is "contributes to cardiovascular health". The target population is assumed to be the general population. The claimed effect has not been sufficiently defined and no clarifications were provided by Member States.

- The claimed effect is general and non-specific, and does not refer to any specific health claim as required by Regulation (EC) No 1924/2006.

Treatment of prostate cancer (ID 3588)

- The claimed effect is "contributes to maintain a healthy prostate and breast". The target population is assumed to be the general population. From the references provided, it is assumed that the claimed effect is related to the treatment of prostate cancer.

- The claim is related to the treatment of diseases and does not comply with the criteria laid down in Regulation (EC) No 1924/2006.

"Upper respiratory tract" (ID 3589)

- The claimed effect is "contributes to the upper respiratory tract health". The target population is assumed to be the general population. The claimed effect has not been sufficiently defined in the information provided.

- The claimed effect is general and non-specific, and does not refer to any specific health claim as required by Regulation (EC) No 1924/2006.

DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 (No: EFSA-Q-2008-1874, EFSA-Q-2008-2024, EFSA-Q-2008-2390, EFSA-Q-2008-2440, EFSA-Q-2008-2873, EFSA-Q-2008-3825, EFSA-Q-2008-3886, EFSA-Q-2008-4312, EFSA-Q-2008-4313, EFSA-Q-2008-4314, EFSA-Q-2008-4315, EFSA-Q-2008-4955, EFSA-Q-2008-4964 , EFSA-Q-2008-05018, EFSA-Q-2008-05019). The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The full list of supporting references as provided to EFSA is available on: http://www.efsa.europa.eu/panels/nda/claims/article13.htm.

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APPENDICES

APPENDIX A

BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation 1924/2006 on nutrition and health claims made on foods[6] (hereinafter "the Regulation") entered into force on 19th January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

a) the role of a nutrient or other substance in growth, development and the functions of the body; or

b) psychological and behavioural functions; or

c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

(i) based on generally accepted scientific evidence; and

(ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13 (1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

ISSUES THAT NEED TO BE CONSIDERED

IMPORTANCE AND PERTINENCE OF THE FOOD[7]

Foods are commonly involved in many different functions[8] of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

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[6] OJ L12, 18/01/2007
[7] The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.
[8] The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).

It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

> (a)   the claimed effect of the food is beneficial for human health,
>
> (b)   a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),
>
> (c)   the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,
>
> (d)   the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

WORDING OF HEALTH CLAIMS

Scientific substantiation of health claims is the main aspect on which EFSA's opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the

requirement to describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/ terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

TERMS OF REFERENCE

HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN'S DEVELOPMENT AND HEALTH

EFSA should in particular consider, and provide advice on the following aspects:

➢ Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.

➢ Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.

➢ The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

➢ the claimed effect of the food in the identified function is beneficial.

➢ a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity consumed.

➢ where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.

➢ the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

➢ the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

➢ on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.

APPENDIX B

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.

APPENDIX C

Table 1. Main entry health claims related to soy isoflavones, including conditions of use from similar claims, as proposed in the Consolidated List.

ID	Food or Food constituent	Health Relationship	Proposed wording
1135	Soya (Glycine max [L.] Merr.)	Cholesterol management / heart health	Inclusion of at least 25g [or 40-90 mg soy isoflavones] soya protein per day as part of a diet low in saturated fat promotes heart health / helps control blood cholesterol.

Conditions of use

− Consumption of at least 25 g of soya protein (or 40-90 mg soy isoflavones) a day, as part of an overall diet low in saturated fat

− 25 g soy protein per day

− Must be at least a source of protein as per Annex Regulation 1924/2006, 25g soya protein per day

− Must include a statement to the affect that at least 25g of soya protein per day must be consumed as part of a diet low in saturated fat. Products carrying this claim should: i) Not imply that consumption of more, or less, than 25g per day is advantageous. ii) Not imply that consumption of 25g soya protein per day is a dietary requirement. iii) Contain a minimum of 5g* of soya protein per serving (*Amended 260903). iv) State what constitutes a serving and the amount of soya protein provided in each serving expressed as grams or millilitres, e.g. 'One 200ml glass'; 'One 125g pot' etc. v) State the proportion (i.e. a 'fifth', 'quarter', 'third', 'half' etc) of the 25g daily intake in each serving, e.g. 'A 100g serving contains 8.34g of soya protein, which is one third of 25g'. vi) The claim relates to soya protein that has retained its naturally occurring isoflavones

ID	Food or Food constituent	Health Relationship	Proposed wording
1286	Soy	Vascular effects including protection from oxidative damage	-soy offers specific vascular benefits; '-soy helps keep the arteries healthy and helps keep a healthy heart; -soy helps protect the body tissues and cells from oxidation oxidative damage.

Conditions of use

− 1-2 servings per day (40mg of isoflavones)

− Intake of 45 mg isoflavones (aglycone equivalent) per day

ID	Food or Food constituent	Health Relationship	Proposed wording
1654	Soy Isoflavones	Menopause	Helps to maintain a calm and comfortable menopause /helps women coping with the telltale signs associated with menopause, such as hot flushes, sweating, restlessness and irritability

Conditions of use

- Products with 80-150mg/100g, 25-50mg/dose of soy isoflavones. The quality and amount of the lactic acid in the gut's microbial flora affect the usefulness of isoflavones and lignan. Isoflavones/dose: 25–50 mg Isoflavones/day (2 doses): 50–100 mg.

- Max 80 mg per day

- Minimum 35 mg per day;

- 35 to 100 mg of soy isoflavones per day

- 50 mg pro Tag

- 100 mg täglich–Nahrungsergänzung

- > 40 mg/d–Erst bei Dosierungen von 40 mg/d und darüber von Genistein und Daidzein oder deren Vorläufermolekülen Biochanin A und Formononetin wurden klinische Effekte nachgewiesen.

- Frauen ab 40 —Standardisierter Extrakt aus Sojakeimen—40mg isoflavone je Einzeldosis—Täglich–

- Frauen ab 40 –Täglich–

- 60 mg/d

- 50 mg / jour

ID	Food or Food constituent	Health Relationship	Proposed wording
1704	Soy Isoflavones	Attività estrogenica. Rinnovamento cutaneo Clarification provided Menopause/ Skin and hair health during menopause/'Cholesterol management	Possono aiutare i capelli della donna in un periodo di naturale cambiamento come il climaterio e la menopausa. Utili per la tonicità della pelle. Per il benessere della donna in età menopausale. . Può aiutare a mantenere i fisiologici livelli di colesterolo. Clarification provided Soy Isoflavones are phytoestrogens that may improve skin tonicity and decrease hair loss during menopause.Soy Isoflavones decreases blood cholesterol levels.

Conditions of use

- 80 mg per day

- Soya extract rich in isoflavones At least 20 mg isoflavones per day

ID	Food or Food constituent	Health Relationship	Proposed wording
2140	Soy Glycine max	Soy contains the phytoestrogens isoflavones that can function as either an estrogen agonist or antagonist	Soy helps maintain well-being during menopause Soy can positively affect the well-being during menopause

		Clarification provided Soy reduces discomfort related to menopause	
Conditions of use			
− Extract equal to 40 mg isoflavones/day			

ID	Food or Food constituent	Health Relationship	Proposed wording
3093	Soya Isoflavones	Act as phyto-estrogens <u>Clarification provided</u> Helps maintain normal cholesterol Menopause: Necessary for a calm and comfortable menopause. Helps women coping with the teltale signsassociated with menopause, such as hot flushes, sweating, restlessness and irritablity.	Soya isoflavones act as/are phyto-estrogens. Contains phytoestrogens Added goodness of soya isoflavones, which act as phytoestrogens. Added goodness of soya phytoestrogens Contains soya isoflavones, which act as phytoestrogens
Conditions of use			
− 20mg isoflavones per serving			

ID	Food or Food constituent	Health Relationship	Proposed wording
3154	isoflavones	helps to keep healthy termoregulation during climacterium	helps to reduce untoward effects of climacterium e.g. hot flush, exudation, strong heartbeat
Conditions of use			
− 50 mg of isoflavones per day			

ID	Food or Food constituent	Health Relationship	Proposed wording
3587	SOIA ISOFLAVONI	Contributes to cardiovascular health	Contributes to cardiovascular health
Conditions of use			
− Dried extract (tit. total isoflavons 40%): 0.8-1 mg/kg/day, divided in 2-3- doses with empty stomach			
No clarification provided by Member States			

ID	Food or Food constituent	Health Relationship	Proposed wording
3588	SOIA ISOFLAVONI	Contributes to maintain a healthy prostate and breast. <u>Clarification provided</u> Acting as phytoestrogenes decrease the effects due to levels of sexual hormones in the blood.	Useful for normal prostate function.

	Conditions of use		
	− Dried extract (tit. total isoflavons 40%): 0.8-1 mg/kg/day, divided in 2-3- doses with empty stomach		

ID	Food or Food constituent	Health Relationship	Proposed wording
3589	SOIA ISOFLAVONI	Contributes to the upper respiratory tract health	Balm: due to its balsamic activity could help during the cool season.

	Conditions of use		
	− Dried extract (tit. total isoflavons 40%): 0.8-1 mg/kg/day, divided in 2-3- doses with empty stomach		
	No clarification provided by Member States		

ID	Food or Food constituent	Health Relationship	Proposed wording
3590	SOIA ISOFLAVONI	Helps to alleviate the symptoms of menopause	Contributes to the female hormonal balance during clymaterium. Helps in case of augmented request of nutrients.

	Conditions of use		
	− Dried extract (tit. total isoflavons 40%): 0.8-1 mg/kg/day, divided in 2-3- doses with empty stomach		
	No clarification provided by Member States		

ID	Food or Food constituent	Health Relationship	Proposed wording
4245	soyfoods (isoflavones)	Antioxidant status	Soyfoods naturally contain antioxidants (isoflavones) which help the body to fight free radicals.

	Conditions of use		
	− Intake of 45 mg isoflavones (aglycone equivalent) per day		

ID	Food or Food constituent	Health Relationship	Proposed wording
4254	Dry isoflavones soya extract	Hair growth and loss	Act on hair bulb in order to support hair growth. Prevent hair from premature ageing via their antioxidant properties and on the microcirculation.

	Conditions of use		
	− 20 mg per day		
	No clarification provided by Member States		

GLOSSARY AND ABBREVIATIONS

8-epi-PGF$_{2\alpha}$	8-epiprostaglandin F$_{2\alpha}$
DNA	Deoxyribonucleic acid
DRI	Daidzein-rich isoflavone aglycones
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
ISP	Isolated soy protein
IU	International Unit
LDL	Low-density lipoprotein
MDA	Malondialdehyde
NCEP	National Cholesterol Education Program
RCT	Randomised controlled trial
ROS	Reactive oxygen species
TAS	Total antioxidant status